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GATING KINETICS AND ION TRANSFER IN CHANNELS OF NERVE
MEMBRANE(U) TEXAS UNIV MEDICAL BRANCH AT GALVESTON DEPT
OF PHYSIOLOGY AND BIOPHYSICS H M FISHMAN 21 OCT 87
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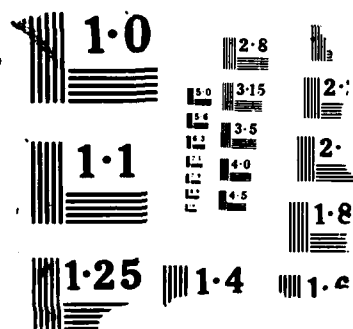
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The ultimate objective of this project is to obtain an electrochemical description of the molecular processes underlying the movement of ions across nerve membranes during excitation of a nerve fiber. The short-term objectives are to obtain fundamental information on the processes of ion transfer in Na and K channels known as "gating" (electric-field initiated alteration of the membrane to the conduction of specific ions) and "conductance" (the passage of ions through a channel after ions gain access to a channel). Whether the gating mechanism can be validly described by linear, equilibrium processes will be determined by examining the time-reversal properties of ion channel conductance fluctuations and/or by comparison of gating-current fluctuations with those predicted from the Nyquist relation and measured membrane admittance. The kinetics of gated channels derived from analysis of stochastic (microscopic) current fluctuations and those derived from macroscopic determinations will be compared to ascertain whether an assumed relationship exists. Furthermore, the macroscopic gating kinetics of Na and K channels derived from step clamp transients will be compared with			
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19. Those derived in steady state via small-amplitude admittance measurements to ascertain if kinetic determinations are time invariant. Conductance studies will focus on the voltage-sensitivity of the Na channel conductance and whether this could arise from channel blockage by Ca^{2+} or H^{+} ions, multiple conducting states of channels, or from a heterogeneous population of channels.

Research Objectives

This project employs innovative measurements and techniques to obtain fundamental electrochemical information on the processes of ion transfer in Na and K channels known as "gating" (electric-field initiated alteration of the membrane to the conduction of specific ions) and "conductance" (the passage of ions through a channel after ions gain access to a channel) in order to assess the applicability of linear versus nonlinear rate theory and equilibrium versus nonequilibrium thermodynamics to models of ion channel kinetics.

Brief statement of major accomplishments in the first year

The focus of activity during the past year was on obtaining meaningful macrokinetic characterization of three types of K channels (outward delayed rectifier, inward anomalous rectifier and Ca-activated K conductance) in three preparations: squid giant axon, neuronal soma membranes in snail (Helix aspersa) and sea hare (Aplysia). In the classical squid axon preparation a detailed comparison between estimates of the relaxation time obtained via Hodgkin-Huxley (HH) analysis of step, voltage-clamp current responses and direct linear analysis in the steady state (200 msec after step clamp) via curve fits of a complex admittance model to acquired admittance data showed that the HH estimates were both model dependent and empirical in that the estimates do not agree with those obtained from a truly linear analysis (admittance). Thus the HH methodology cannot be used to obtain kinetic parameters that can be related to any physicochemical theory. Alternatively, we have shown that characterization of the linear kinetics of ion conduction processes in membranes is more appropriately carried out via admittance measurements and analysis. Admittance measurements were thus used exclusively to obtain relaxation times of K conductances in the neuronal preparations.

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Progress ReportAnalysis of K Conductance Relaxations in Squid Axon Membrane

Application of the Hodgkin-Huxley (HH) power law formulation has become a standard procedure in the analysis of conduction in membrane preparations. Whether the HH methodology and formulation yields relaxation times that are independent of the empiricism of the assumed power law and whether the relaxation times are consistent with those obtained by a direct (small signal) linear analysis were previously unexamined questions. To resolve these issues, intact squid axons were voltage clamped by conventional axial electrode techniques and the K current response recorded after blockage of Na conduction by tetrodotoxin (1 μ M) added to the external artificial sea water. The K current response to step voltage clamps from 1 to 80 mV from a holding potential of -65 mV were fitted by the equation

$$i_K = I_{K_0} (1 - K_0 e^{-t/\tau_n})^x \quad (1)$$

where I_{K_0} and K_0 are arbitrary parameters and τ_n , the relaxation time, and x , the exponent in the HH power law formulation are variables which could be changed in order to minimize (least squares criterion) the difference between equation (1) and the current records with leakage and capacitive transient removed. The best fits to data, as seen in Fig. 1, were obtained for x variable rather than 4, the HH value. Thus, estimates of τ_n depended on the value of x used to fit the step response, as shown in Fig. 2. Clearly, the relaxation times determined in this way are an empirical set of numbers.

Next, we carried out a driving point (admittance) linear analysis to obtain $\tau_n(V)$ directly and to compare the values obtained with those from an HH analysis of step clamp responses in the same axon. Rapid acquisition of admittance data was achieved by use of a Fourier synthesized signal as a small perturbation

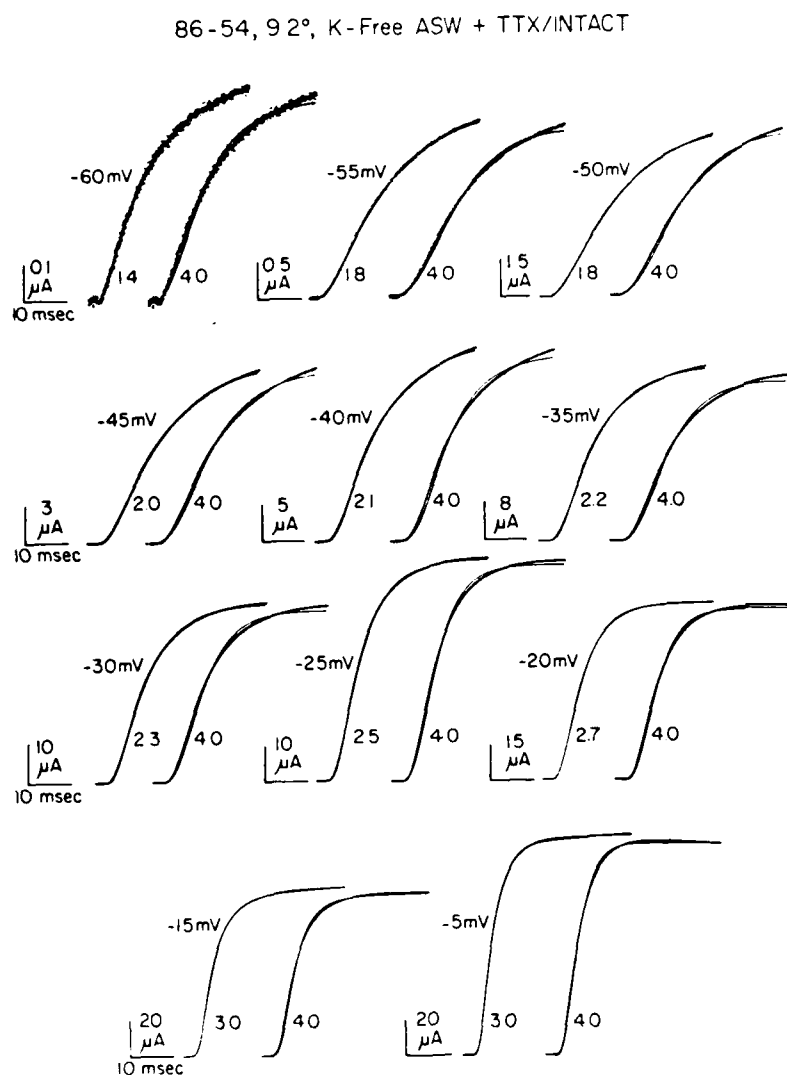


Fig. 1 Comparison of the Hodgkin-Huxley (HH) model fits of eq. (1) of the squid axon K current (capacitive transient and leakage current removed) in response to 5 mV (-60 mV) to 60 mV (-5 mV) voltage steps from a holding potential of -65 mV.

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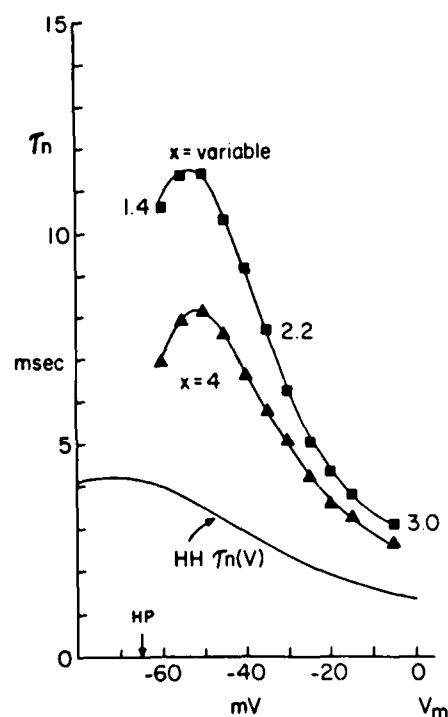


Fig. 2 K conductance relaxation time as a function of voltage, $\tau_n(V)$, derived from best fit of the HH description of i_K in eq. (1) for $x = \text{variable}$ and $x = 4$ to squid axon, voltage clamp responses in Fig. 1. HH $\tau_n(V)$ curve is calculated from HH formulation for 9.2°, assuming a $Q_{10} = 3$. Curves fits of data by eye.

superposed on a step voltage clamp together with Fourier techniques developed by us and described previously. Admittance data acquired 200 msec after each step clamp was shown to be in steady state and were fitted by the equations

$$Y(j\omega) = [R_s + Y_m^{-1}(j\omega)]^{-1} \quad (2)$$

where R_s is the resistance between voltage-sensing electrodes and the inner and outer membrane surfaces and $Y_m(j)$ is the membrane complex admittance, given by

$$Y_m(j\omega) = (j\omega)^\alpha C_m + g_o + g_k (1 + j\omega\tau_n)^{-1} \quad (3)$$

In equation (3), C_m is membrane capacitance, α is a number < 1 that reflects the constant-phase-angle character of C_m , g_o is the membrane chord conductance, and the frequency-dependent term involving g_k and τ_n reflects the relaxation kinetics.

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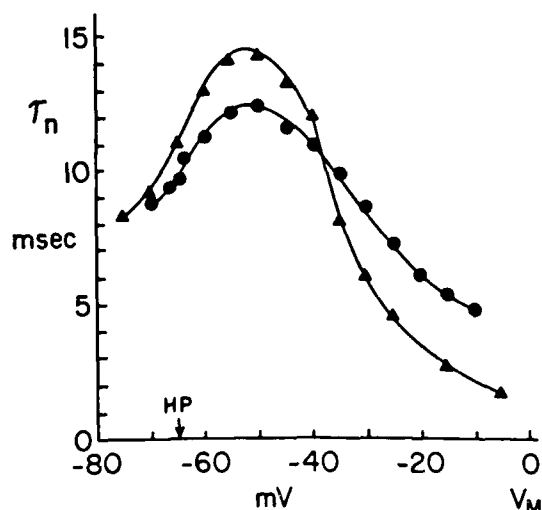


Fig. 3 Comparison of K conductance relaxation times, τ_n , determined by model curve fits of : (1) step voltage clamp responses with x variable (filled circles), as in Fig. 1 and (2) the membrane admittance 200 msec after step clamps (filled triangles) in the same axon and under the same experimental conditions. Curves are drawn by eye.

Fig. 3 shows the $\tau_n(V)$ data obtained by fit of equations (2) and (3) to squid axon admittance data (filled triangles) together with the estimates obtained from fit of equation (1) to step clamp transient responses with x variable (the best fit) in the same axon. We conclude that an HH analysis is not equivalent to a linear analysis and that it yields values for τ_n that have

no relationship to kinetic parameters outside of the HH formulation. Furthermore, characterization of the linear kinetics of ion conduction via fits of admittance data have the following distinct advantage over an HH analysis: values of n do not depend on the empirical exponent x , corrections for series resistance are not required and removal of capacitive transient and leakage are not necessary.

Linear Analysis of K Conduction from Impedance Determinations in Snail Neuron

Three types of potassium conductance were distinguishable pharmacologically in isolated single neuron from Helix aspersa (snail). The linear kinetic parameters of the voltage-sensitive, "delayed rectifier" K conductance and the Ca-activated, K conductance were obtained by curve fitting model admittance functions of frequency to complex admittances obtained from the reciprocal of measured impedance data. The complex impedance of the K conduction process of an identified soma cell membrane was measured in the frequency range 0.25 Hz to 100 Hz. Relaxation times of activation of voltage-sensitive, "delayed rectifier" K conductance were in the range 14.7 msec at 0 mV and 6 msec at 40 mV. Relaxation times for the Ca-activated, K conductance were estimated to be between 108 msec at 0 mV and 65.7 msec at 50 mV. The capacitive-like kinetic behavior of the delayed rectifier decreased from $1.67 \mu\text{F}/\text{cm}^2$ to $0.64 \mu\text{F}/\text{cm}^2$ in this depolarized voltage range. At hyperpolarized voltages in high external potassium, a small amount of rectification of inward K current was observed. A manuscript containing the details of this work has been accepted for a special issue of the journal FERROELECTRICS.

Inward K Rectifier Channel Kinetics from Analysis of Complex Conductances in Aplysia Neuronal Membrane.

The inward K^+ rectifier in Aplysia neuron and Ba^{++} blockade of the rectification process were studied by rapid measurement of complex admittances during voltage clamps to membrane potentials in the range of -90 to -40 mV. Complex ionic conductances of K^+ and Cl^- rectifiers were extracted from complex admittances of other membrane conduction processes and capacitance by vector subtraction of the membrane complex admittance during suppressed inward K^+ current (near zero-mean current and in zero $[K^+]_o$) from complex admittances determined at other $[K^+]_o$ and membrane potentials. The contribution of the K^+ rectifier to the admittance is distinguishable in the frequency domain above 1 Hz from the contribution of the Cl^- rectifier, which is only apparent at frequencies less than 0.1 Hz. The voltage dependence (-90 to -40 mV) of the chord conductance (0.2 to 0.05 μS) and the relaxation time (4-8 ms) of the K^+ rectifier at $[K^+]_o = 40$ mM were determined by curve fits of admittance data by an admittance model based on the linearized Hodgkin-Huxley equations. The conductance of the inward K^+ rectifier at -80 mV membrane potential had a square-root dependence on external K^+ concentration and the relaxation time increased from 2 ms to 7.5 ms for $[K^+]_o = 20$ and 100 mM, respectively. The admittance of the inward K^+ rectifier, affected by Ba^{++} , was obtained by complex vector subtraction of the admittance for which the inward K^+ rectifier was blocked (at -35 mV and $[Ba^{++}]_o = 5$ mM) from admittances determined at -80 mV and at other Ba^{++} concentrations. The relaxation time of the blockade process decreased with increases in Ba^{++} concentration. An open-closed channel state model produces the inductive-like kinetic behavior in the admittance of the inward K^+ rectifier and the addition of a blocked channel state accounts for the capacitive-like kinetic behavior of the Ba^{++} blockade process. A manuscript

detailing this work has been submitted for publication in the Biophysical Journal.

Additional activities

A manuscript was submitted for publication in a book on the "Mechanistic approaches to Interactions of Electromagnetic Fields with Living System". The main thesis of the paper is that the responsiveness of elasmobranch fishes to weak electric fields can be understood from the known anatomical structures and electrophysiology of the electroreceptive organ known as the ampulla of Lorentzini in these fishes. Within each ampulla there are 1-10 thousand receptor cells which respond to the same electric field and whose outputs are summed in the afferent nerve which innervates the basolateral surface of these receptor cells. By summation of their outputs the noise level of any one receptor is reduced by the factor $1/\sqrt{n}$, where n is the number of receptor cells.

Publications

1. Fishman, H.M. and Lipicky, R.J. 1987. Transient and steady state analysis of K conductance relaxations in squid axons. Biophys. J. 51:51a.
2. Fishman, H.M. and Lipicky, R.J. 1987. HH analysis of K current relaxations does not yield the relaxation time of the linearized conducting system. 9th International Biophysics Congress, Jerusalem, Israel.
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Manuscripts Submitted for Publication

1. Hayashi, H. and Fishman, H.M. 1987. Inward K rectifier channel kinetics from analysis of complex conductances in Aplysia neuronal membrane. Biophys. J.

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